Molecular basis of cell migration in the fish lateral line: Role of the chemokine receptor CXCR4 and of its ligand, SDF1

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Cell migration plays an essential role in many morphogenetic processes, and its deregulation has many dramatic consequences. Yet how migration is controlled during normal development is still a largely unresolved question. We examined this process in the case of the posterior lateral line (PLL), a mechanosensory system present in fish and amphibians. In zebrafish, the embryonic PLL comprises seven to eight sense organs (neuromasts) aligned from head to tail along the flank of the animal and is formed by a primordium that originates from a cephalic placode. This primordium migrates along a stereotyped pathway toward the tip of the tail and deposits in its wake discrete groups of cells, each of which will become a neuromast. We show that a trail of SDF1-like chemokine is present along the pathway of the primordium and that a CXCR4-like chemokine receptor is expressed by the migrating cells. The inactivation of either the ligand or its receptor blocks migration, whereas in mutants in which the normal SDF1 trail is absent, the primordium path is redirected to the next, more ventral *sdf1* expression domain. In all cases, the sensory axons remain associated to the primordium, indicating that the extension of the neurites to form the PLL nerve depends on the movement of the primordium. We conclude that both the formation and the innervation of this system depend on the SDF1-CXCR4 system, which has also been implicated in several migration events in humans, including metastasis formation and lymphocyte homing.

The lateral line is a sensory system that allows fish and amphibians to perceive and localize movements in their vicinity (1). It is involved in important behaviors such as prey detection, predator avoidance, or swimming in schools. The individual sense organs, called neuromasts, are arranged on the body surface in species-specific patterns and are innervated by bipolar sensory neurons located in a cranial ganglion. The formation of this system is peculiar in that the neuromasts originate from cephalic placodes, yet end up being spread all over the body. This dispersal occurs through a set of stereotyped, long-range migration events. The development of the lateral line provides therefore an excellent opportunity to analyze the developmental control of cell migration.

A conspicuous component of the system is the lateral row of neuromasts that extends from head to tail along the horizontal myoseptum and gave the "lateral line" system its name. This row belongs to the posterior lateral line (PLL), as opposed to the rows on the head, which form the anterior lateral line. The anterior and posterior lines differ in the location of their placode of origin (anterior and posterior to the otic placode, respectively), in the location of their ganglia (anterior and posterior to the ear, respectively), and in the position of their central projection in the hindbrain.

At the end of zebrafish embryogenesis, the PLL comprises seven to eight regularly spaced neuromasts aligned along the horizontal myoseptum (Fig. 1*A*). All neuromasts are laid in place by a primordium that migrates all of the way from the postotic placode to the tip of the tail and deposits in its wake small groups of cells. Each group will subsequently differentiate as a neuromast (2). The embryonic pattern is progressively completed during postembryonic growth through reiterations of the embryonic process, with new primordia migrating along the same path and forming additional neuromasts that intercalate between the embryonic organs (3, 4).

In addition to the migrating primordium, the PLL placode also generates the PLL sensory neurons. The first neurons differentiate shortly before the primordium begins its migration (5). They extend two axons, a central one into the hindbrain and a peripheral one into the primordium. As the primordium begins to migrate, sensory growth cones remain associated with it and pull the axons all of the way to the tail, thereby forming the PLL nerve (2). It has been suggested that the axons are guided independently of the primordium by the repulsive effect of a semaphorin expressed in the dorsal and ventral regions of the somites (6).

Recent work (5, 7) has shown that the deposition of neuromasts results from a process of partitioning that is intrinsic to the primordium. This partitioning relies on the activation of proneural and neurogenic genes in a manner that is reminiscent of the formation of mechanosensory organs in insects (7). Once defined through the local expression of proneural genes, the future neuromast slows down until it eventually stops completely and differentiates (5). This change in behavior is accompanied by changes in the expression of genes coding for membrane proteins. One of them is down-regulated in the cells of the future neuromast and might therefore be involved in controlling the migratory properties of the cells (5). This gene has recently been described as *cxcr4b* (8) and encodes a homolog to the human chemokine receptor CXCR4.

Here, we describe the expression of *sdf1a*, a gene that codes for a peptide homologous to SDF1, the chemokine that binds to and activates CXCR4. We show that the SDF1-CXCR4 system drives the migration of the primordium and defines its stereotyped pathway. We also show that aberrant migration of the primordium results in aberrant axonal pathways, suggesting that the axons are primarily guided by the primordium rather than by other cues. Given its simplicity, resolution, and accessibility, the zebrafish PLL might be a favorable system to explore further the SDF1–CXCR4 interaction.

Materials and Methods

Fish Maintenance. Zebrafish were maintained as described by Westerfield and coworkers (9). The mutants *slow-muscle-*

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Abbreviations: hpf, hours postfertilization; PLL, posterior lateral line.

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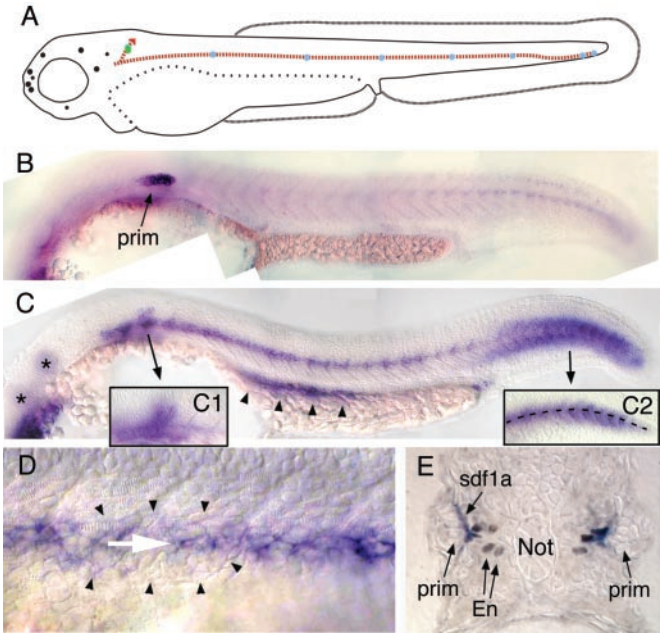

Fig. 1. Migration of the PLL primordium. (*A*) In a 2-day-old embryo, the neuromasts of the PLL (blue dots) are deposited by a primordium that migrates along a stereotyped path (red dotted line). The first neuromast of the dorsal branch (green dot) has just formed; this line will later extend toward and along the dorsal midline (red arrowhead). (*B*) At 24 h postfertilization (hpf), the gene *cxcr4b* is predominantly expressed in the migrating primordium. (*C*) At the same time, the gene *sdf1a* is predominantly expressed in a stripe of cells that marks the pathway that the primordium will follow. Labeling is also observed at the level of the pronephros (arrowheads), rhombomeres 4 and 6 (asterisks), and in the forebrain. (*C1*) At 29 hpf, there is a marked expression of *sdf1a* at the level of somite 1, prefiguring the path of the dorsal branch. (*C2*) At 29 hpf, the posterior expression of *sdf1a* has narrowed down to the ventral portion of the terminal somites. (*D*) The PLL primordium (outlined by arrowheads) follows precisely the path marked by *sdf1a*-expressing cells in its posterior-wards migration (gray arrow). (*E*) In a section at the level of the migrating primordia (prim), the expression of *sdf1a* (blue) corresponds to a subset of myoseptal *engrailed*-expressing cells (En, gray nuclei). Not, notochord.

omitted (*smu*) and *chameleon* (*con*) were obtained from S. Wilson and the Tübingen Stock center.

***In Situ* Hybridization.** *In situ* hybridizations and combined *in situ* hybridization–immunolabeling were performed according to ref. 10.

Labeling of the Neuromasts. In some cases the neuromasts were visualized by *in situ* hybridization with a specific probe identified by P. Mourrain and F. Rosa in a screen for Nodal inducible RNAs (P. Mourrain and F. Rosa, unpublished results). This probe is freely available. In other cases the neuromasts were labeled with 4-(4-diethylaminostyryl)-*N*-methylpyridinium iodide (4-Di-2-Asp, Sigma D-3418) as described in ref. 11.

Morpholino-Antisense Gene Inactivation. Morpholino oligos were obtained from GeneTools (Philomath, OR) and injected at a concentration of 0.5 mM (*sdf1a*) or 1.5 mM (*cxcr4b*). Morpholino sequences were as follows: *sdf1a*-Mo, 5′-ATCACTTTGAGATC**CAT**GTTTGCA; and *cxcr4b*-Mo, 5′-ATGATGCT.ATCGTAAAATTC**CAT**TT. In both cases, the **CAT** sequence corresponding to the start ATG is in bold. The efficiency of the *sdf1a*-Mo was assessed in an *in vitro* translation assay. The translation of *sdf1a* mRNA is reduced in the presence of 400 nM *sdf1a*-Mo and abolished at 4 μM, whereas the translation of *gfp* mRNA is unaffected at 4 and 40 μM *sdf1a*-Mo (not shown). The eight bases at the 5′-end of the *cxcr4b*-Mo correspond to the cDNA rather than to the genomic sequence, as there is an intron at the position marked by a period. Morpholinos corresponding to sequences extending further 3′ could not be used because of internal folding. The presence of the intron probably explains why the frequency of *cxcr4b*-Mo mutant phenotypes is relatively low ($\approx$50%); yet the high specificity of the mutant phenotype and its similarity to the *sdf1a*-Mo phenotype make us confident that it reflects *cxcr4b* inactivation.

Labeling of the Sensory Neurons. Embryos were fixed in paraformaldehyde 4% (vol/vol) in PBS for at least one night at room temperature. Fixed embryos were mounted in agar (0.7% in PBS) on small (7 $\times$ 7 mm) coverslips placed on normal glass slides and viewed under Nomarski optics on a fixed stage (FS) Axioscop microscope with a $\times$40 long-distance water-immersion objective. They were impaled with a pulled glass electrode backfilled with DiI C18 (Molecular Probes), 2 mg/ml in dimethylformamide, and a small amount of dye was delivered in the ganglion by passing current with an electrometer (World Precision Instruments, Sarasota, FL). The coverslips with agar and embryo were transferred to PBS-filled wells and left overnight before being examined under epifluorescence on a Nikon Microphot FXA equipped with a Princeton Pentamax camera.

Results

***cxcr4b* Is Expressed in the PLL Primordium.** The PLL primordium appears at 20 hpf and follows a stereotyped course in its journey to the tip of the tail (Fig. 1*A*), which it reaches at 40 hpf (12). Two expressed sequences have recently been reported to be heterogeneously distributed in the primordium (5). One of them, CB 403, is present at high levels in the migrating cells (Fig. 1*B*) and is down-regulated in the cells that will be deposited. This sequence corresponds to the gene *cxcr4b*, one of the two zebrafish homologs to the human chemokine receptor gene CXCR4 (8), and codes for a protein which is 63% identical to the human receptor. We examined more closely the onset of *cxcr4b* expression and found that the gene is already expressed at 20 hpf, as soon as the PLL primordium can be detected. At 18 hpf, however, there is no expression posterior to the otic vesicle. We conclude that the onset of *cxcr4b* expression coincides with the appearance of a migrating primordium in the postotic region. Besides its expression in the PLL, *cxcr4b* is noticeably expressed in the rhombencephalon in a highly dynamic pattern, in the forebrain, and in the eye (not shown).

***sdf1* Is Expressed Along the Myoseptum.** We have identified two zebrafish genes encoding proteins related to the human chemokine SDF1, the ligand of the CXCR4 receptor, through a search in EST libraries. We will refer to them as *sdf1a* and *sdf1b*. Both genes are 44% identical to the human gene at the amino acid level and are 73% identical to each other. The gene *sdf1b* is not expressed along the prospective path of the PLL primordium, except in a broad domain in the tail region (not shown), and was not investigated further. In contrast, *sdf1a* is prominently expressed in a stripe of cells at the level of the horizontal myoseptum (Fig. 1*C*) and defines exactly the pathway that the primordium will follow (Fig. 1*D*). At the time the primordium begins its journey (20 hpf), *sdf1a* is expressed along the anterior one-half of the myoseptum; this expression progressively extends posteriorly over the next few hours (Fig. 1*D*). In the most posterior tenth of the embryo, *sdf1a* is initially expressed in a broad region, but the expression shifts to the ventral one-half of the tail (Fig. 1*C Inset C2*) at 29 hpf, a time at which the primordium is approximately midway in its journey.

This pattern of expression defines exactly the pathway of the PLL primordium, which follows the horizontal myoseptum over

most of its course but moves to a more ventral trajectory at the level of somite 27 (Fig. 1*A*). At 29 hpf, an oblique stripe of *sdf1a* expression extends dorsally at the level of the first somite (Fig. 1*C Inset C1*). This path is precisely the one that will be followed by the primordium of the dorsal line, which originates at ≈38 hpf, migrates in a dorsal direction (4) and founds the dorsal branch of the PLL, which will later extend along the dorsal midline (Fig. 1*A*).

The horizontal myoseptum corresponds to a slab of *engrailed*-expressing myoblasts (Fig. 1*E*, gray nuclei). Although the expression of *sdf1a* occurs at the same dorso-ventral level, and concerns some of the *engrailed*-expressing cells, it is strictly confined to the superficial layer (Fig. 1*E*, blue). In addition to this stripe, *sdf1a* is also expressed in other places, notably in mesenchymal tissue located between ectoderm and pronephros (Fig. 1*C*, arrowheads) and in the CNS, in the hindbrain (rhombomeres 4 and 6, asterisks), in the forebrain, and in the distal part of the optic stalk, regions where cell migrations play a prominent role in mammals.

Inactivation of *sdf1a* and *cxcr4b* Prevents Primordium Migration. To examine whether *sdf1a* is involved in primordium migration, we inhibited its translation by using the morpholino-antisense approach. At 32 hpf, the primordium has reached the level of the anus in WT embryos (Fig. 2*A*). In *sdf1a* morphants, however, the primordium has moved little or not at all (Fig. 2*B*; 95% $n = 40$). Control injections made with anti-*admp* morpholinos (13) did not produce any abnormality in the PLL pattern (Fig. 2*C*; $n = 73$). At 52 hpf, when the PLL normally comprises a line of 7–8 neuromasts (Fig. 2*D*), the treated embryos show no or very few neuromasts (Fig. 2*E*; 92% $n = 13$). The neuromasts of the head, however, form normally. We conclude that *sdf1a* is an essential component of the migration of the PLL primordium.

We similarly assessed the involvement of *cxcr4b* in primordium migration by morpholino-antisense gene inactivation. At 3 days, a time when the embryonic line is complete, we observed major defects in the PLL in approximately one-half of the injected embryos (47% $n = 17$). Defective lines ranged from the presence of a single neuromast just behind the ear (Fig. 2*F*) to a few neuromasts not extending beyond the anus. The variability of phenotypes is likely caused by the fact that the first intronic junction of *cxcr4b* is very close to the start ATG (see *Materials and Methods*). As in the case of *sdf1a* inactivation, the pattern of neuromasts on the head is normal and serves as a control of the generally good condition of the treated embryos. Control injections with a morpholino that corresponds to the *cxcr4b* sequence just downstream of the start AUG gave no PLL phenotype (Fig. 2*G*; 100% $n = 19$). We conclude that *cxcr4b* is an essential component of the migration of the embryonic PLL primordium. We also observed that the eyes are reduced in size in approximately one-half of the morphant (Fig. 2*F Inset*). We have not attempted to look for defects in the rhombencephalon or forebrain.

Formation of the PLL in Mutants That Lack a Myoseptum. The myoseptum derives from paraxial mesoderm under the influence of signals emanating from notochord, including hedgehog-related molecules. The *slow-muscle-omitted* (*smu*) is a zebrafish gene related to the *Drosophila* gene *smoothened*, a receptor for hedgehog (9, 14). The development of the notochord is normal in embryos mutant for *smu*/*smoothened*, but the horizontal myoseptum does not form, presumably because the paraxial mesoderm cannot process notochord-derived hedgehog signaling (15). Mutant *chameleon* (*con*) embryos also lack a horizontal myoseptum (16). The origin of the defect is not known, but the overall phenotype is very similar to that of *smu*/*smoothened* embryos, including normally differentiated notochord and somitic tissues.

The *smu*/*smoothened* and *con* mutant embryos fail to express *sdf1a* in the medial region of the somites, but they maintain the expression at the level of the mesenchyme abutting the pronephros (Fig. 2*H*; $n = 47$). We observed that, in both mutants, the primordium migrates along this alternative pathway (Fig. 2*I*; $n = 11$), albeit at a slower pace. At 3 days, three to four neuromasts have been deposited to form an aberrant, ventrally located PLL (Fig. 2*J*; $n = 17$). Because the primordium path is redirected to the next *sdf1a* expression domain in mutants, we conclude that *sdf1a* acts as a guidance cue.

Axonal Pathways in Morphants and Mutants. The neuromasts are innervated by sensory neurons whose cell bodies are clustered in a cranial ganglion and project in the rhombencephalon (Fig. 3 *A* and *B*). Sensory growth cones invade the primordium before it begins to migrate and remain associated with it during its journey to the tip of the tail (2). It has been proposed that the axons are guided along the myoseptum independently of the primordium, by the repulsive effect of a semaphorin, SemaZ1a, which is expressed in the dorsal and ventral regions of the somites but not in the middle section (6). To examine the behavior of the axons both in morphants and in mutants, we labeled the sensory neurons by applying DiI to the PLL ganglion.

In *sdf1a* morphants, we observed that the growth cones remain associated to the now immobile primordium (Fig. 3 *C* and *D*; $n = 7$), suggesting that the sensory axons are guided by the primordium rather than by other cues. We also examined the pathway followed by the axons in mutants where the PLL forms ectopically. As shown above, the *smu* and *con* mutations prevent the formation of the horizontal myoseptum, resulting in the formation of an ectopic PLL. We labeled the PLL ganglion of 32-hpf mutant embryos and observed that the axons follow an aberrant ventral course that closely follows that of the primordium [shown in Fig. 3*E* for *smu*; ($n = 5$) and Fig. 3*F* for *con* ($n = 5$)]. At later times (Fig. 3 *G* and *H*; $n = 3$), the axons of *con* mutant embryos establish synaptic connections with the aberrantly positioned neuromasts (compare to Fig. 2*I*). We also noted that the dorsal branch does not form in the mutants (Fig. 3*H*, compare to Fig. 3*B*).

The mutations *smu* and *con* might alter the distribution of SemaZ1a and affect the axonal pathways independently of their effect on the primordium. We examined the expression of *semaZ1a* in both mutants and compared it to the WT pattern (Fig. 3*I*). We found that the expression of *semaZ1a* is modified in *smu* mutants (Fig. 3*J*), which is consistent with the idea that the lack of expression of *semaZ1a* along the horizontal myoseptum requires hedgehog signaling from the notochord (6). However, the expression of *semaZ1a* is not detectably affected in *con* mutants (Fig. 3*K*). Because the axons follow similar ectopic courses in *smu* and *con* mutants (Fig. 3 *E–H*), irrespective of the fact that the pattern of expression of *semaZ1a* is altered in one mutant but not in the other (Fig. 3 *J* and *K*), we conclude that the distribution of SemaZ1a plays no role in defining the course of the PLL axons.

Discussion

Our results suggest that the expression pattern of *sdf1a*, a zebrafish homolog to the human chemokine SDF1, defines the stereotyped pathway that the PLL primordium will follow. We have also shown that in the absence of SDF1a, the primordium fails to migrate or moves for very short distances. Thus the trail of SDF1-like chemokine along the horizontal myoseptum is an essential component of PLL primordium migration. CXCR4b, a putative receptor of the SDF1a chemokine, is expressed by the migrating cells of the primordium. The onset of *cxcr4b* expression takes place between 18 and 20 hpf; i.e., it coincides with the onset of primordium migration, whereas the arrest of migration (deposition of neuromasts) corresponds to the down-regulation

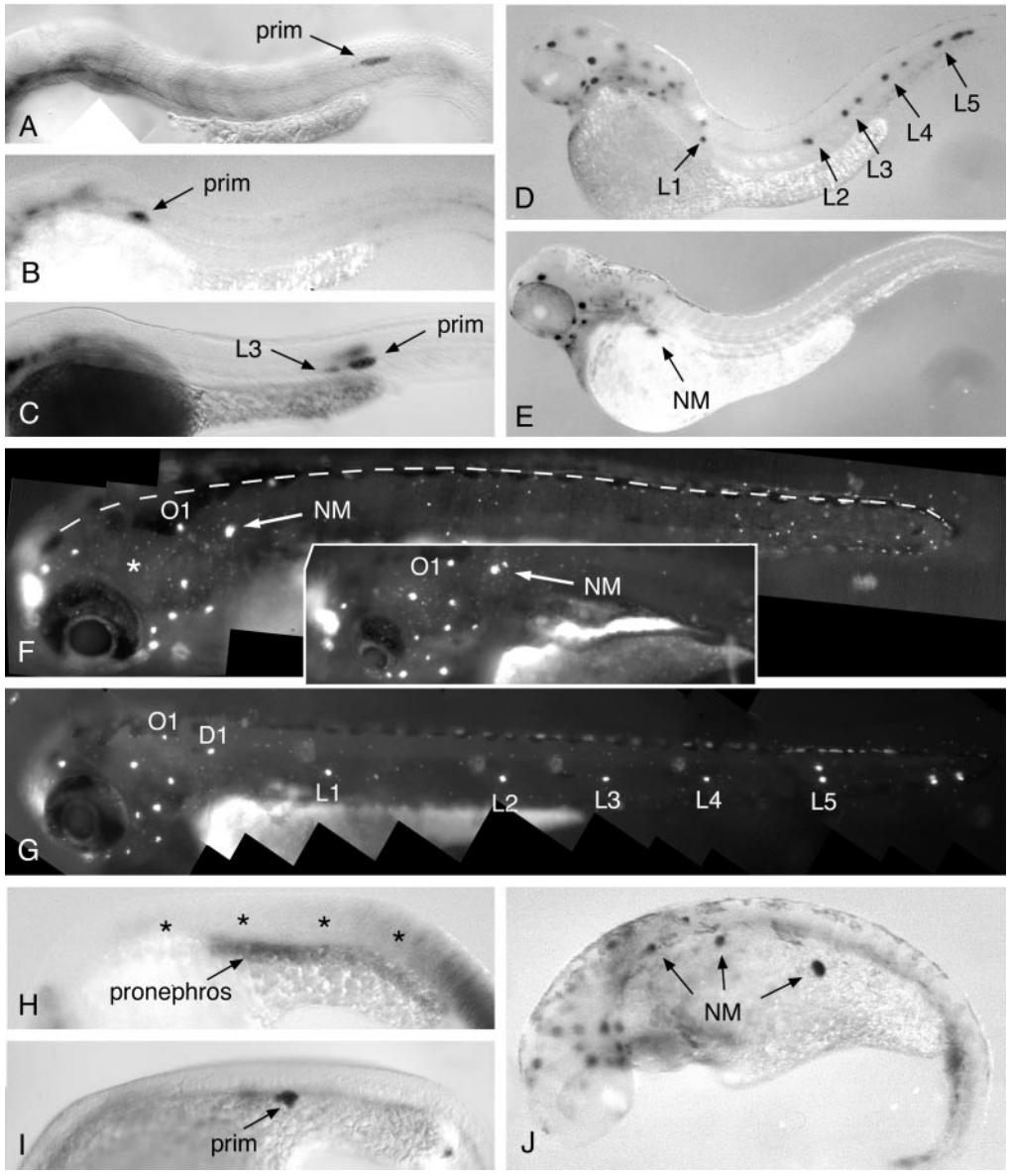

Fig. 2. Morphants and mutants for *sdf1a* and *cxcr4b*. (*A*) The position of the PLL primordium ≈32 hpf in a normal embryo. The primordium has reached the level of the anus and is approximately halfway through its migration. In this and the following panels, the primordium is labeled by the *cxcr4b* probe. (*B*) In a *sdf1a* morphant (where *sdf1a* has been inactivated by morpholino-antisense injection), the primordium has barely moved at 32 hpf. (*C*) In a control embryo injected with a different morpholino, the primordium migrates normally. In this particular embryo, a group of cells is being deposited; note that *cxcr4b* is clearly down-regulated in these cells. (*D*) Pattern of neuromasts in a 2-day-old normal embryo. The neuromasts of the PLL are marked L1–L5; the two terminal neuromasts are not marked. (*E*) In a 2-day-old *sdf1a* morphant embryo, a single neuromast (NM) has formed at an abnormal, very anterior position. The head neuromasts show a normal pattern. In both *D* and *E*, neuromasts are stained with a specific probe (see *Materials and Methods*). (*F*) A 3-day-old *cxcr4b* morphant embryo labeled with 4-(4-diethylaminostyryl)-*N*-methylpyridinium iodide (4-Di-2-Asp), a fluorescent marker that specifically labels the hair cells of the neuromasts. The PLL is reduced to a single large neuromast behind the ear (NM); the occipital neuromast (O1) as well as the head neuromasts have formed normally. In this particular embryo, one supraorbital neuromast is missing (asterisk). The dorsal midline is marked by a dashed line. (*Inset*) Anterior one-half of another 3-day-old *cxcr4b* morphant embryo with a small eye; in this embryo, the PLL was reduced to two closely apposed, very anterior neuromasts (NM). In both cases, the ''NM'' neuromasts are located just posterior to the ear and anterior to the first somite, near the position occupied by the first neuromast of the dorsal line, D1, in normal embryos. (*G*) A 3-day-old control embryo that had been injected with a morpholino that recognizes the *cxcr4b* sequence just downstream of the start ATG. The PLL comprises five neuromasts aligned along the horizontal myoseptum, L1–L5, two terminal neuromasts at the tip of the tail, and the first neuromast of the dorsal line, D1. The occipital neuromast O1 has also been indicated to facilitate the comparison with the morphant embryos. (*H*) The *smu* mutant embryos lack a horizontal myoseptum and loose the lateral stripe of *sdf1a* expression (asterisks, compare to Fig. 1*C*). The expression of *sdf1a* is maintained at the level of the pronephros. (*I*) In a 32-hpf *smu* embryo, the PLL primordium (labeled with the *cxcr4b* probe) migrates along an ectopic ventral course that corresponds to the region where *sdf1a* is expressed. However, the movement is much slower than normal; compare to *A*. (*J*) In a 72-hpf *smu* embryo, three to four neuromasts are deposited and form an ectopic, ventral PLL. Same probe as in *C* and *D*.

of this gene in trailing cells of the primordium (5). Thus, the expression of *cxcr4b* is tightly correlated to the migratory behavior of the cells. We conclude that the migration of the PLL primordium is predominantly determined by the interaction between the SDF1 ligand, which determines the path, and its CXCR4 receptor, which controls the movement.

The observation that the primordium closely follows the trail of *sdf1a* expression suggests that the chemokine acts mostly over

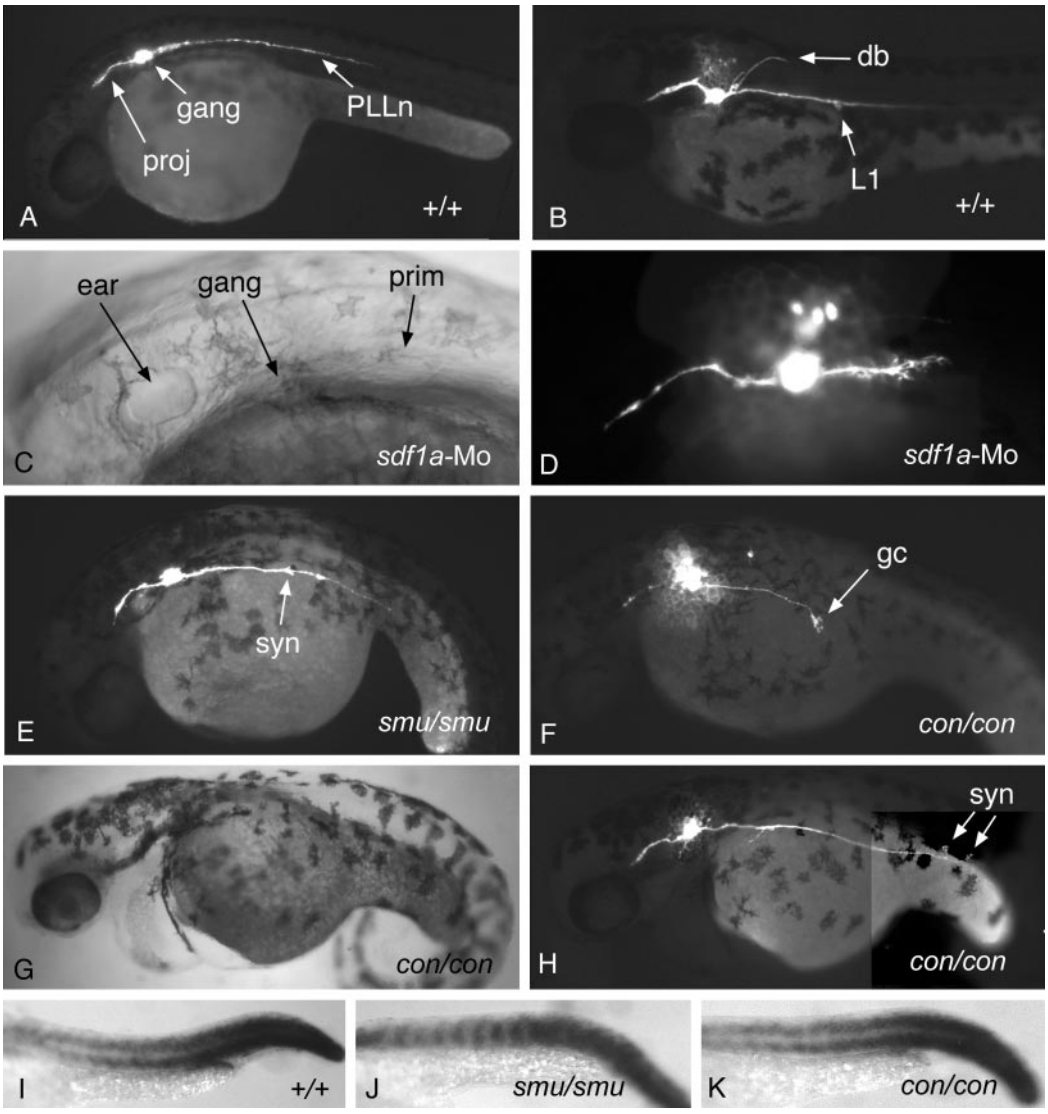

Fig. 3. Sensory neurons in morphants and mutants. (*A*) Application of the lipophilic neuronal tracer DiI in the PLL ganglion (gang), in fixed 32-hpf embryos. The central projection in the hindbrain (proj) and peripheral nerve extending to the primordium (PLLn) are labeled. (*B*) At 52 hpf, a synaptic specialization has developed under the L1 neuromast and the dorsal branch (db) has formed. (*C*) In a 32-hpf *sdf1a* morphant, the primordium (prim) has remained immobile, just posterior to the ganglion (gang). The otic vesicle (ear) has developed normally. (*D*) In the same embryo, the sensory neurons have extended a normal projection in the hindbrain, just beneath the ear, but their peripheral neurites have not extended beyond the primordium, where they branch profusely. (*E*) In a 32-hpf *smu* embryo, where the primordium travels along an ectopic ventral course, the sensory neurites follow this new pathway. A synapse with an ectopic neuromast (syn) is beginning to form in this particular embryo. (*F*) In *chameleon* (*con*) embryos, the horizontal myoseptum also fails to form and the corresponding stripe of *sdf1a* expression is missing. As in *smu* embryos, the sensory neurites accompany the primordium along its new course. gc, growth cones extending in the migrating primordium. (*G*) A 52-hpf *con* embryo. (*H*) The PLL neurons labeled in this embryo extend along the ectopic, ventral course of the primordium and establish synapses (syn) with two ''terminal'' neuromasts. (*I*) Expression of *semaZ1a* in a 24-hpf WT embryo. The gene is expressed in two broad domains dorsal and ventral to the horizontal myoseptum, as described (6). The expression has disappeared by 30 hpf (not shown). (*J*) In a 24-hpf *smu* embryo, the expression of *semaZ1a* does not show the normal gap at the level of the horizontal myoseptum. (*K*) In *con* embryos, however, the pattern of *semaZ1a* expression is not detectably modified.

a short range. Consistent with this conclusion, we never observed cases where a primordium would be attracted by the more ventral stripe of *sdf1a* expression, at the level of the pronephros, except in mutants where the myoseptum expression is abolished. Thus, the pathway of the primordium seems to be determined by short-range diffusion of the SDF1a product, suggesting that the latter may be bound locally to the extracellular matrix on being secreted. Because there is no indication of a gradient of SDF1a concentration from anterior to posterior, we assume that the trail of *sdf1a* expression determines the pathway, but not the direction of migration. A similar conclusion has been reached for the development of the PLL in the axolotl, based on the effect of rotating pieces of epidermis on the path of the primordium (17). In this case also, it was observed that the underlying mesodermal tissue must be intact for migration to proceed normally.

Our analysis of PLL sensory neurons revealed that the growth cones of the sensory axons remain in intimate contact with the primordium even when the latter does not move, or moves along an aberrant pathway. Therefore, we conclude that the sensory axons are guided by the primordium, and that the formation of the PLL nerve ultimately depends on the SDF1-CXCR4 system. This finding contrasts with data suggesting that the neurites are driven along the myoseptum by the repulsive action of a sema-

phorin, SemaZ1a, which is present in dorsal and ventral regions of the somites (6). Our observation that the pathways followed by PLL axons in *smu* and *con* reflect the ectopic course of the primordium, irrespective of the fact that the pattern of expression of *semaZ1a* is altered in one mutant but not in the other, supports the conclusion that the distribution of SemaZ1a plays no role in defining the course of the PLL axons.

One possible interpretation of this discrepancy is that SemaZ1a may have a repulsive effect on both primordium and axons, such that its ectopic expression along the normal SDF1a pathway could lead to disruptions in the migration of the primordium and, eventually, to ectopic axonal pathways. Because in normal situations *semaZ1a* is not expressed in a broad region surrounding and including the *sdf1a* trail, this repulsive effect would normally play no role in primordium or axonal guidance but may act as some sort of "back-up" system and ensure a rough guidance in case the normal *sdf1a/cxcr4b* mechanism would fail. Whether SemaZ1a misexpression can alter the course of the primordium has not been directly examined, however (6).

The guidance of the sensory growth cones by the primordium has been reported to depend on the HNK-1 epitope, which is highly expressed by the PLL neurons. Blocking HNK-1 results in axons being diverted to aberrant courses, including into SemaZ1a territory, although the migration of the primordium is not affected (18). One possible mechanism for axonal guidance is, therefore, an HNK1-promoted binding of the growth cones to neural cell adhesion molecule (NCAM). Of interest, NCAM is expressed both by the primordium cells and by the axons themselves, and could, therefore, be involved both in guiding the pioneer PLL neurons and in fasciculation guidance of later growing fibers (18, 19).

The SDF1-CXCR4 system has been implicated in various instances of cell migration in mammals, including the migration of lymphocytes and the formation of metastases (20). Developmental defects presumably related to altered migration have also been reported in the CNS of *cxcr4* knock-out mice (21–23). The prominent expression of *sdf1a* and *cxcr4b* in regions of the fish brain where migrations are known to play an important developmental role, such as in the rhombencephalon, are also suggestive of a role in the control and patterning of neuronal cell migration. Taken together, the data suggest an evolutionarily conserved role for this ligand-receptor system in very diverse contexts.

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